

## "Genetic Factors Affecting Susceptibility to Low-Dose Radiation"

DOE project # ER62859 0005021

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**Rationale:** Risk estimates for radiation exposure are based on the whole population. There is evidence that there may be subpopulations within the human population that are genetically susceptible to ionizing radiation, e.g., patients with the disorders of Nijmegen Breakage Syndrome (NBS) and Ataxia telangiectasia (AT). These patients are radiation sensitive because of defects in cellular responses to radiation induced genetic damage. It is unclear whether humans heterozygous for the mutations associated with NBS or AT are radiation sensitive and results from cell culture experiments give conflicting results. NBS and AT patients are easily identified but obligate heterozygotes for these disorders, estimated to be 1-4% of the US population, may be susceptible to low doses of radiation.

**Hypothesis tested:** That animals with genetically defined haploinsufficiency at the NBS1 loci are predisposed to radiation sensitivity.

**Experimental design:** Two unique mice models with engineered mutations in the NBS1 gene were exposed to 0, 1, 10 or 100cGy of X-rays 8 weeks after birth and monitored for delayed radiation effects. This was a life time study, and we used analysis of micronuclei to determine whether hypomorphic animals showed increased evidence of radiation-induced genomic instability and tumor induction to determine potential sensitivity.

**Results:** Analysis of micronuclei was used to investigate potential delayed genomic instability occurring in irradiated animals. At regular intervals post irradiation blood samples were taken from all animals using tail vein sticks, and the frequency of micronuclei per 2000 polychromatic erythrocytes determined. There were no significant differences in micronuclei frequencies between irradiated and non-irradiated animals, or between wild type and our hypomorphic NBS mice. We did however, notice a small, but not statistically significant increase in the frequency of micronuclei as the animals age.

There was also no increase in tumor incidence between wild type and heterozygous animals after irradiation. Animals were followed until they died from natural causes or in rare cases had to be euthanized upon advice of the animal care staff because of animal discomfort. There was no non-specific life shortening associated with low dose radiation exposure in either wild type or heterozygous animals when compared with the non irradiated control animals, and if anything the 10cGy irradiated NBS1<sup>+/-</sup> animals lived longer than both the non-irradiated control and NBS1<sup>+/-</sup> animals.

We also used these animals to determine if we could develop a rapid and sensitive assay for identifying individuals heterozygous for NBS in the human population. Our goal was to develop a functional assay by which heterozygotes can be distinguished from DNA polymorphisms in order to identify potentially sensitive individuals. We used both qualitative and quantitative Mre11/Rad50/NBS1 foci formation as a function of radiation dose and could never reliably identify NBS heterozygotes despite detailed genomic analysis. We also investigated the cytogenetic G2 assay as a method of identifying NBS<sup>+/-</sup> individuals but we did not find a consistent or statistically significant difference in chromatid aberrations between NBS<sup>+/+</sup> and NBS<sup>+/-</sup>. Animals with two mutant NBS alleles were identifiable using this technique, but this is obviously not relevant to the human situation.

**Conclusions:** Mice genetically engineered to show the same mutation in one allele of the NBS1 gene as the vast majority of NBS patients do not exhibit enhanced low dose radiation sensitivity as measured by delayed genomic instability or tumor induction.